Heavy Metals in the Environment

A Novel Continuous-Flow Sequential Extraction Procedure for Metal Speciation in Solids

J. Shiowatana,* N. Tantidanai, S. Nookabkaew, and D. Nacapricha

ABSTRACT

A continuous-flow extraction system was developed to speed up, facilitate, and improve the accuracy of the chemical fractionation of metals in solid materials. A three-step sequential extraction scheme was used to evaluate the novel system by analyzing calcium (Ca), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) in a soil certified reference material (National Institute of Standards and Technology [NIST] SRM 2710). In the proposed system, extraction occurred in a closed chamber through which extractants were passed sequentially. The extracts were collected in a number of subfractions for subsequent flame atomic absorption analysis. Apart from the advantages of simplicity, speed, and less risk of the contamination that flow analysis systems usually possess, the continuous-flow system can improve the accuracy of chemical fractionation of metals by sequential extraction. The system ensures that extraction is performed at designated pH values without any need of adjustment. Variation of sample weight to chamber volume ratios from 1:12 to 1:40 had no effect on the extractability of the metals studied. In the extraction of the acid soluble fraction, concentrations of acetic acid in the range 0.11 to 0.5 mol L^{-1} had no significant effect on the amounts of metals extracted, except Fe. Increasing the concentration of hydroxylamine in the reducible fraction step from 0.04 to 0.5 mol L^{-1} affected the extraction efficiency for Fe, Mn, and Zn. The extraction profile, rather than a single value of extracted concentration, of each element offers additional information about the kinetics of leaching processes and chemical associations between elements in the solid materials.

SEQUENTIAL extraction to fractionate metals or other elements in solid materials (soils, sediments, sludge, solid wastes, etc.) into several groups of different leachability is widely employed to determine the distribution of metals in different phases. Although the procedures used are generally tedious and time consuming, the results furnish detailed information about the origin, mode of occurrence, bioavailability, potential mobility, and transport of the metals in natural environments. The technique is therefore widely used as a tool for the study of the origin and fate of metals in the environment. However, despite the fact that the development and use of sequential extraction schemes started in the early 1970s, uniformity in the procedures used is still lacking. In addition, problems of poor selectivity, redistribution during extraction, and the dependency of results on operating conditions have been frequently raised.

The most widely used sequential extraction schemes are probably those proposed by Tessier et al. (1979) and the Community Bureau of Reference (BCR) (Quevauviller, 1998). These procedures have been demonstrated to give satisfactory results for the targeted phases owing to a careful selection of reagents and detailed operating conditions. However, many authors (Kheboian and Bauer, 1987; Shan and Chen, 1993; Tu et al., 1994; Raksasataya et al., 1996; Lo and Yang, 1998) have reported the problem of readsorption and the dependence of readsorption on the phase composition of the sample being analyzed. Kheboian and Bauer (1987) discovered that the recovery of spiked metals (Pb, Zn, Cu, Ni) was less for model mixed-phase sediments than for single-phase materials, and questioned the selectivity of the sequential extraction procedure. Some authors (Howard and Shu, 1996; Raksasataya et al., 1997) have attempted to counteract readsorption. For example, chelating agents have been added with the extractant to prevent readsorption and thus improve the accuracy of the extraction. They recommended that the extraction times of the earlier steps of sequential extractions should be kept as short as possible so as to minimize the opportunity for readsorption to take place.

Being operationally defined procedures, sequential extractions inevitably give results that are dependent on the extraction parameters such as type, concentration, and pH of each reagent, sample weight to extractant volume ratios (S/E), extraction times and temperatures, methods of shaking and phase separation, etc. The effect of extraction conditions as potential sources of poor reproducibility was investigated (Sahilquillo et al., 1999) using the BCR's three-step sequential extraction procedure to analyze a certified reference material. The authors found that the final pH of the extractant in the reducible metal extraction step could differ from its original pH by up to 1.5 pH units. The pH change during extraction was dependent on the composition of the sample and affected the amount of metal leaching considerably. Davidson et al. (1999) also indicated that differences in extraction pH were an important source of variability in analytical results. Some proposed procedures, which emphasize the need to adjust the pH of extractants during extraction, should therefore not be overlooked.

The effect of extractant concentration has also been studied (Sahilquillo et al., 1999; Maiz et al., 1997) and found to affect extractability in both the exchangeable

---

J. Shiowatana and D. Nacapricha, Trace Element Lab., Institute of Science and Technology Research and Development, and J. Shiowatana, N. Tantidanai, S. Nookabkaew, and D. Nacapricha, Dep. of Chemistry, Faculty of Science, Mahidol Univ., Rama VI Road, Bangkok 10400, Thailand. Received 28 Feb. 2000. *Corresponding author (scysw@mahidol.ac.th).

Published in J. Environ. Qual. 30:1195–1205 (2001).

Abbreviations: BCR, Community Bureau of Reference (European Community); FAAS, flame atomic absorption spectrometry; S/C, sample weight to chamber volume ratio; S/E, sample weight to extractant volume ratio.

and acid-soluble metal extraction steps. Varying the S/E ratio from 1:5 to 1:50 has been found to affect extractability of some metals but not others (Shan and Chen, 1993; Sahilquillo et al., 1999). Extraction temperature has also been found to affect the extraction efficiency for some metals (Sahuquillo et al., 1999).

The effect of extraction time on metal extractability has been reported not to be crucial, probably due to the relatively long extraction time employed in most procedures. Maiz et al. (1997) proposed a rapid sequential extraction procedure by reducing the extraction time in their metal bioavailability study of contaminated soils. Perez-Cid et al. (1996) used an ultrasonic probe to reduce the extraction time from 16 h to 7 min. Other researchers have recommended the use of a minimal extraction time to allow a steady state to be established between sample and extractant, but not so long that dissolution of other phases can occur (Kennedy et al., 1997). Finally, small operational variations in factors such as speed of centrifugation, shaking mode, and solid–liquid phase separation procedures have also been reported to cause noncomparability of results (Sahuquillo et al., 1999; Quevauviller et al., 1993).

Many attempts are being made toward achieving comparability and standardization of sequential extraction procedures by concerned institutions. Among them are the International Standard Organization (ISO), which coordinates working groups on standardization of analytical methods and suppliers of certified reference materials who may include or have already included (Ure et al., 1993; Nagourney et al., 1997) certified values for the phase distribution of some metals. In addition, the European Commission's Standard, Measurement and Testing Program (former BCR) (Quevauviller, 1998; Quevauviller et al., 1998) has launched a project to bring together those concerned to join discussions leading to harmonized and agreed procedures.

In this work, a novel approach will be presented to improve the accuracy and comparability of sequential fractionation by solving many of the problems described above. Instead of using a conventional batch extraction process, a continuous-flow extraction process has been used. Although Hirner (1992) made a suggestion that a dynamic extraction process in a flow cell could help improve the extraction accuracy through reduction of readsorption effects, no previous reports have been made of any investigations using this approach.

A continuous-flow extraction system was developed and a three-step sequential extraction procedure was used to test the system. The BCR scheme for metal speciation was modified in this study because of its less-complicated nature and rather wide acceptance. For the first two steps of the sequential extraction, acetic acid and hydroxylamine hydrochloride are used to leach acid-soluble and reducible fractions, respectively. For the oxidizable fraction, a mixture of hydrogen peroxide and nitric acid is used to oxidize the targeted phase, followed by leaching of the oxidized products with ammonium acetate solution. It is not practical to follow such a procedure exactly in a continuous-flow extraction system. Therefore, extraction was performed using hydrogen peroxide in nitric acid alone. Since there have been reports questioning the completeness of extraction at ambient temperature, the effect of temperature and other parameters such as extraction pH, reagent concentration, and sample weight to chamber volume ratio on the extraction efficiency have also been investigated.

MATERIALS AND METHODS

Extraction Chamber and Setup

An extraction chamber was designed to allow containment and stirring of a weighed sample. Extractants could flow sequentially through the chamber and leach metals from the

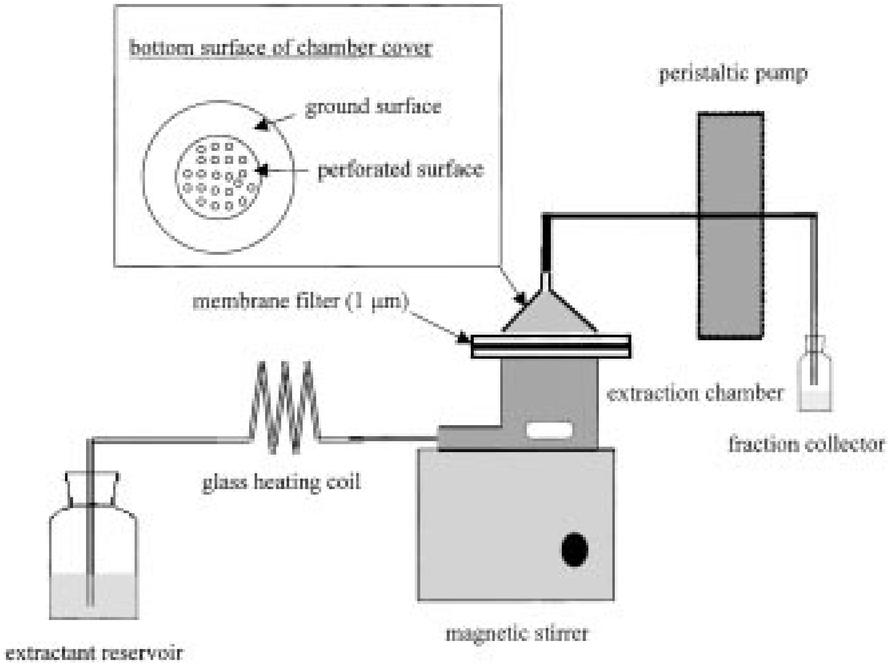

Fig. 1. Diagram of a continuous-flow system.

targeted phases. Chambers and their covers (Fig. 1) were constructed from borosilicate glass to have a capacity of approximately either 3 or 10 mL. The outlet of the chamber was furnished with a filter (Whatman [Maidstone, UK] glass microfibre filter GF/B, 47-mm diameter, 1-μm particle retention) to allow dissolved matter to flow through. Extractant was pumped through the chamber using a peristaltic pump (Ismatec [Glattbrugg-Zürich, Switzerland] MS-CA4/620C) at its highest rate using silicone tubing of 2-mm inner diameter (flow rate for an aqueous solution is approximately 10 mL min^{-1}). Heating of extractants, when required, was carried out by passing extractant through a glass heating coil approximately 1 m in length, which was wrapped in a winding heating tape (Glas-Col [Terre Haute, IN] 300 watt).

Extraction in a Continuous-Flow System

A continuous-flow extraction with an on-line detection system was initially investigated using the extraction chamber, as in Fig. 1. The outlet from the chamber was directly connected to the nebulizer of a flame atomic absorption spectrometer (FAAS). An extraction profile was obtained by plotting the analytical signal against extraction time. Since the flow rate of extractant to the FAAS was not uniform and decreased with time due to gradual blockage of the filter by fine solid particles, the peak area of the extraction profile could not be directly related to the amount of metal extracted. An off-line system was therefore preferred for this study because it could give additional advantages.

In the off-line system, the extract was collected in constant volume subfractions. The subfractions were subjected to subsequent determination of metal concentration and other required measurements. The remaining extracts can be kept for later use, unlike the on-line system where extracts are totally consumed by the FAAS measurement. In addition, the operation time of the spectrometer is much less in the off-line system. The extraction profile, which will be referred to as an *extractogram* hereafter, is obtained by a graphical plot of concentration of metals against subfraction number. In an off-line system, the volume of each subfraction will affect the number of subfractions to be collected. With smaller subfraction volumes, a larger number of subfractions and a more detailed extractogram is obtained, although a longer detection time is required.

In this study, flame atomic absorption spectrometric detection could be used owing to the high level of metals in the sample investigated. More sensitive methods will be needed for the detection of some trace elements in uncontaminated soils. A FAAS (PerkinElmer [Norwalk, CT] Model 3100) equipped with a deuterium background corrector was used for the determination of metals. Concentrations of metals were obtained by the standard addition technique.

Preparation of Standard Solutions and Glassware

Ultrapure water from a MilliQ water purification unit (Millipore, Bedford, MA) was used throughout. All glassware used was previously cleaned and soaked in 10% HNO_3 and rinsed with pure water. Standard metal solutions (1000 μg mL^{-1}) were purchased from Merck (Darmstadt, Germany) or prepared in house from pure metals. Working standard solutions were prepared by diluting the stock solution with ultrapure water when needed.

Solid Materials and Extraction Reagents

A soil certified reference material, National Institute of Standards and Technology (NIST) SRM 2710, was used. The material was a highly contaminated soil from Montana, USA. It was carefully prepared to achieve a high degree of homogeneity, and certified concentration values of a number of elements were given. It was used in this study to provide a convenient way for evaluation of the accuracy of the proposed method and to allow for further comparison of results. The highly contaminated soil was used in order to obtain extracts in which most elements studied could be detected in all extraction steps.

A three-step sequential extraction was carried out using the following solutions:

Step I: Solution A (0.11 mol L^{-1} acetic acid)
Step II: Solution B (0.10 mol L^{-1} hydroxylamine hydrochloride in nitric acid, pH 2)
Step III: Solution C (8.8 mol L^{-1} hydrogen peroxide adjusted to pH 2 with 1 mol L^{-1} HNO_3).

Sequential Extraction Procedure

A weighed sample (0.25 g or as otherwise stated) was transferred to a clean extraction chamber together with a magnetic bar. A glass microfibre filter was then placed on the outlet followed by a rubber gasket, and the chamber cover was securely clamped in position. The chamber was connected to the extractant reservoir and the collector vial using silicone tubing and placed on a magnetic stirrer. The magnetic stirrer and peristaltic pump were switched on to start the extraction. Extract from the continuous flow extraction chamber was collected in plastic vials at required volume intervals. When a total volume of approximately 160 mL was collected, the next extractant was passed through and collection of subfractions was repeated until all three leaching steps were completed. Between Steps II and III, pure water (ca. 20 mL) was passed through the chamber to reduce the vigorous reaction between the two extractants. The extracts were subjected to FAAS measurement after all extraction steps were finished. For the highly contaminated soil, concentration of metals in subfractions of extracts differed widely, therefore some subfractions of extract had to be diluted (2–10 times) before FAAS determination. When a hot extractant was required, an electrical voltage of 40, 50, or 60 V was supplied to the heating tape to obtain an extractant temperature of 50 ± 5, 65 ± 5, or 80 ± 5°C, respectively.

Total Dissolution of Sample and Dissolution of Residue

Closed-system digestion vessels from UNISEAL (Haifa, Israel) of 20 mL capacity were used for acid digestion of soil or sediment samples. Weighed solid samples (0.250 g) or residues from the extraction chamber were transferred to the vessels together with 5 mL HNO_3 and 5 mL HF. The vessels were then tightly sealed and heated in an oven at 150°C for 16 h. After cooling, the digested clear solutions were made up to volume in 50-mL volumetric flasks.

RESULTS AND DISCUSSION

Batch Extraction vs. Continuous Flow Extraction

In a batch extraction procedure, extraction is performed until equilibrium is reached for metals between the solid sample and the extracting solution. However, Vela et al. (1994) indicated that in some cases, most elements might not achieve equilibrium concentration at the end of the shaking time used. This may be another

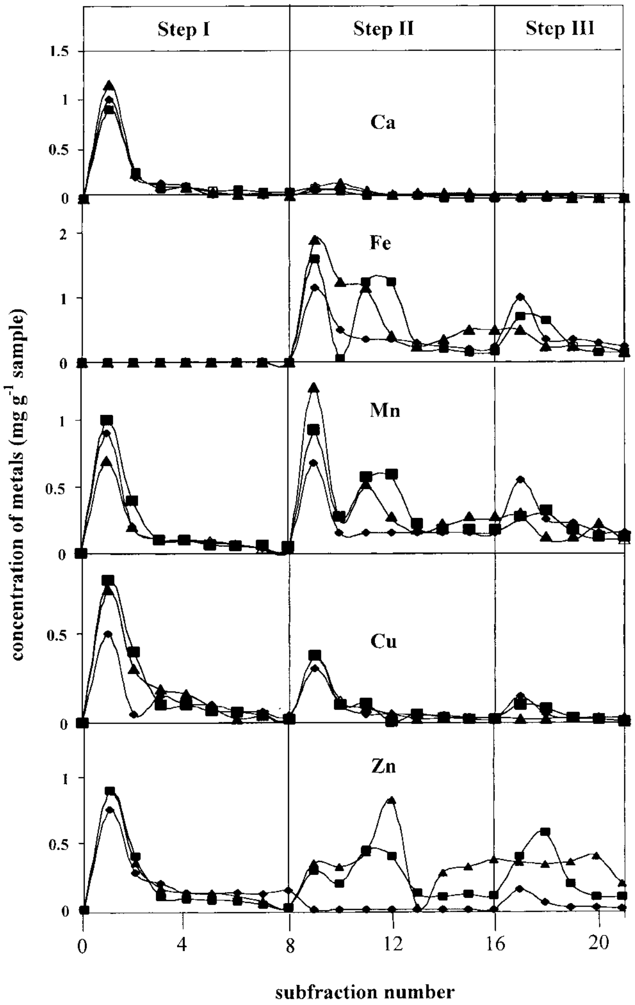

Fig. 2. Extractograms at varying Step II temperatures for a 0.25-g sample of SRM 2710 soil with a 10-mL extraction chamber volume; subfraction volume, 20 mL; ambient temperature (◆), 65°C (■) and 80°C (▲). Temperatures of Steps I and III were kept constant at ambient and 55°C, respectively.

cause of poor reproducibility of the batch extraction technique. In a batch system, the metal extracted may not represent the total amount extractable. The amount extracted is dependent on the solubility limits that different metal ions have in equilibrium with other ions in the extracting solution.

The continuous-flow extraction is principally an exhaustive extraction process because fresh extractant is continuously passing through the sample until the metal in the targeted phase is completely leached, as seen from the signal gradually leveling off to background level. However, in some steps for some samples, the signal was found to become steady with no sign of it reaching background level (Fig. 2, Fe and Zn traces). This is probably due to the slow solubilization of nontargeted phases. In such cases, the extraction was stopped when the signal was less than 10% of the signal at peak maximum, or when no further change in signal was observed.

Different extraction schemes show wide variations in extraction conditions, although the conditions used are usually claimed as specific for the targeted phase and cause the least effect on nontargeted phases. Table 1 shows the differences between two widely used extraction schemes: the BCR scheme and Tessier's scheme. It is interesting to investigate the effect of extraction conditions on extractability, and examine how continuous-flow extraction can be more advantageous in term of lesser vulnerability to extracting conditions. The extraction conditions investigated include temperature, pH, extractant concentration, and sample weight to chamber volume ratio (S/C).

Effect of Extraction Temperature

The effect of extraction temperature on Step II only was performed for the following reasons. Both the BCR and Tessier schemes (see Table 1) use ambient temperature for the extraction of the acid-soluble fraction. In all reports, this fraction has always been carried out at ambient temperature for fear that other phases could be leached at elevated temperatures. Surprisingly, Mahan et al. (1987) reported that no significant difference in extractability was evident when microwave-accelerated extraction was compared with the conventional procedure for all three steps of acid soluble, reducible, and oxidizable metal extraction for the National Bureau of Standards (USA) SRM 1645 sediment sample.

In the current study, the temperature of this first step was maintained at ambient for the above reasons. For Step III, a higher temperature should help to speed up the oxidation reaction and is therefore desirable. However, the temperature at this step could not be performed at a temperature higher than 55°C in the flow system because of vigorous gas evolution, which caused undesirable backpressure in the system. Therefore, extraction was kept at 55°C for Step III.

The extraction temperature of Step II differs between the BCR and Tessier schemes; the BCR scheme using an ambient temperature and Tessier's using an elevated temperature of 85°C. It has been reported (Sahuquillo et al., 1999) that temperature can substantially affect extractability at this step. The results of varying the temperature of Step II for the proposed flow system are presented in Fig. 2 for soil sample SRM 2710. It can be seen from the extractograms that higher temperatures resulted in faster rates of leaching (sharp rise in peaks) and in larger amounts being extracted (larger peak areas). The double peak of Step II (Fig. 2) at elevated temperature may have resulted from the solubilization of two different phases of amorphous and crystalline iron–manganese oxides. It was reported (Luo and Christie, 1998) that extraction of the reducible fraction was not complete if performed at ambient temperature. Leleyter and Probst (1999) extracted amorphous iron oxide at ambient temperature and its crystalline form at 80°C. The temperature difference between the BCR and Tessier schemes for this step has been shown to result in lower amounts extracted by the BCR scheme compared with the Tessier scheme (Usero et al., 1998). From the extractogram in Fig. 2, it can be seen that the use of 65°C can separate the reducible phase into two

Table 1. Comparison of extraction conditions for two commonly used schemes.

Conditions	BCR scheme†	Tessier's scheme‡
1. pH and reagent concentration	Step I: AcOH 0.11 *M* Step II: $NH_2OH{\cdot}HCl$ 0.1 *M*, pH 2 Step III: H_2O_2 8.8 *M*, pH 2–3; NH_4OAc 1 *M*, pH 2	Step I: AcONa/AcOH 1 *M*, pH 5 Step II: $NH_2OH{\cdot}HCl$ 0.04 *M* in 25% AcOH Step III: 3 mL 0.02 *M* HNO_3; 5 mL 30% H_2O_2, pH 2; 3 mL 30% H_2O_2, pH 2; NH_4OAc 3.2 *M* in 20% (v/v) HNO_3
2. Temperature and time	Step I: ambient, 16 h Step II: ambient, 16 h Step III: ambient, 1 h (H_2O_2); 85°C, 2 h (H_2O_2); 85°C, 1 h (H_2O_2); ambient, 16 h (NH_4OAc)	Step I: ambient, 5 h Step II: 96 ± 3°C, 6 h Step III: 85 ± 2°C, 2 h (H_2O_2); 85 ± 2°C, 2 h (H_2O_2); ambient, 30 min (NH_4OAc)
3. Sample weight to extractant volume ratio	Step I: 1 g/40 mL Step II: 1 g/40 mL Step III: 1 g/10 + 10 mL (H_2O_2); 1 g/50 mL (NH_4OAc)	Step I: 1 g/8 mL Step II: 1 g/20 mL Step III: 1 g/11 mL (H_2O_2) 1 g/11 + 5 mL (NH_4OAc)

† BCR, Community Bureau of Reference (European Community); from Sahuquillo et al., 1999.
‡ From Tessier et al., 1979.

peaks believed to be amorphous and crystalline oxide phases, respectively. Ambient temperature was unable to completely extract the reducible phase. Use of 80°C compared with 65°C did not have much effect on the extractability of Ca, Mn, and Cu but affected Fe and Zn. The reason for this can be seen from the extractograms for Fe and Zn that show further leaching occurring on prolonged extraction at 80°C. This resulted in higher amounts of Fe and Zn extracted at Step II and lower amounts extracted in the following step. This effect can be observed in both the extractograms (Fig. 2) and on the amounts of metals extracted (Table 2).

Table 2 shows the sum of the amounts of metals in all subfractions collected at each step at three different extraction temperatures at Step II, while the temperatures of the other steps were kept constant. The amounts extracted are found to be higher at higher extraction temperatures for all elements studied. This agrees with Sahuquillo et al. (1999), who found improved extractability for Pb, Ni, Cu, and Zn when the temperature was raised from 20 to 26 and 40°C.

The results obtained from this study suggested that temperature should be selected carefully in this type of operationally defined fractionation. The phases being attacked at different temperatures may be different, leading to varying extractabilities. The results from this work show that a temperature of 65°C should be used at this step for improved extractability and for selective extraction of amorphous and crystalline oxides phases without attacking the nontargeted phases. This temperature was used in the following experiments.

Effect of the pH of Extraction

The pH of an extraction can seriously affect the leachability of metals, especially for the extraction of acid-soluble fractions. For this reason, the pH of the final solution in a batch process should be measured and brought to the designated extraction pH (Quevauviller et al., 1993). Failure to maintain the pH of extraction can lead to erroneous results especially in the case of highly acidic or alkaline samples. In a continuous-flow system, the pH of extraction in the earlier subfractions can differ from the designated value but gradually approaches and reaches the designated pH when the leaching is near to completion (Fig. 3).

If not measured and carefully adjusted, the extraction pH in a batch process can be substantially different from

Table 2. Extractable amount of Ca, Fe, Mn, Cu, and Zn using continuous-flow sequential extraction at varying temperatures of Step II for SRM 2710 soil. Chamber volume, 10 mL; subfraction volume, 20 mL. Detection limits for Ca, Fe, Mn, Cu, and Zn are 0.14, 0.18, 0.07, 0.03, and 0.03 mg L^{-1} extract, respectively, or 89, 115, 45, 19, and 19 μg g^{-1} soil, respectively (when metals are assumed to be equally distributed in the total extract volume of each step).

Element	Temperature (Step II)	Amount extracted				Total amount	Certified total value
		Step I	Step II	Step III	Residue		
		mg g^{-1} sample ± 2 SD†				mg g^{-1}	
Ca	ambient	1.77 ± 0.06	0.38 ± 0.06	ND‡	9.8 ± 0.2	12.0 ± 0.13	12.5 ± 0.3
	65°C	1.57 ± 0.06	0.27 ± 0.02	ND	9.4 ± 0.2	11.2 ± 0.12	
	80°C	1.84 ± 0.28	0.37 ± 0.10	ND	10.1 ± 0.4	12.3 ± 0.29	
Fe	ambient	ND	3.39 ± 0.28	2.62 ± 0.56	26.6 ± 4.6	32.6 ± 2.7	33.8 ± 1.0
	65°C	ND	5.16 ± 0.64	1.68 ± 0.02	26.7 ± 2.8	33.5 ± 1.7	
	80°C	ND	6.92 ± 1.66	1.32 ± 0.10	27.4 ± 4.4	35.6 ± 2.7	
Mn	ambient	1.61 ± 0.20	1.65 ± 0.24	1.44 ± 0.44	5.60 ± 0.20	10.30 ± 0.29	10.1 ± 0.4
	65°C	1.86 ± 0.04	2.63 ± 0.02	1.98 ± 0.38	4.20 ± 0.16	10.70 ± 0.21	
	80°C	1.49 ± 0.12	2.60 ± 1.02	0.91 ± 0.20	4.98 ± 0.12	9.90 ± 0.53	
Cu	ambient	1.13 ± 0.06	0.69 ± 0.01	0.26 ± 0.02	0.87 ± 0.08	2.95 ± 0.05	2.95 ± 0.13
	65°C	1.61 ± 0.02	0.84 ± 0.01	0.17 ± 0.02	0.49 ± 0.16	3.11 ± 0.08	
	80°C	1.59 ± 0.06	0.88 ± 0.03	0.09 ± 0.02	0.42 ± 0.12	2.98 ± 0.07	
Zn	ambient	1.40 ± 0.20	0.48 ± 0.32	1.47 ± 0.16	3.60 ± 0.20	6.95 ± 0.23	6.95 ± 0.09
	65°C	1.64 ± 0.02	1.57 ± 0.16	1.26 ± 0.06	2.40 ± 0.16	6.87 ± 0.12	
	80°C	1.59 ± 0.16	2.58 ± 0.22	1.26 ± 0.10	2.04 ± 0.12	7.47 ± 0.16	

† *n* = 3.
‡ Not detectable.

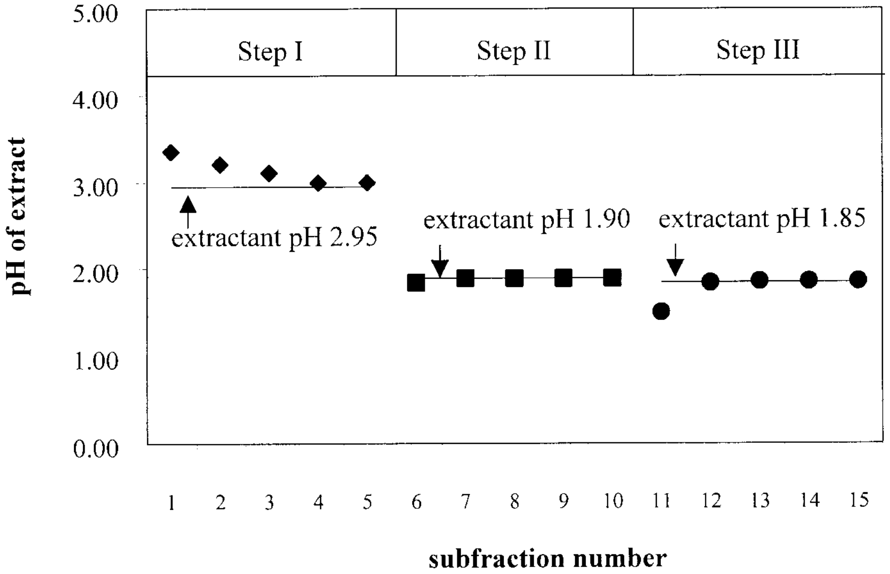

Fig. 3. Change of pH of the extract during continuous-flow extraction. Sample, 0.25 g SRM 2710 soil; chamber volume, 10 mL; subfraction, 20 mL.

the nominal value. Table 3 shows the results of multiple batch extractions of the same sample shown in Fig. 3 without any pH adjustment during extraction. It can be seen that the pH still deviated slightly from the nominal value even in the third batch of the multiple batch process. Better control of extraction pH at the nominal value should lead to better accuracy and precision of extraction in a flow system.

Effect of Reagent Concentration

The effect of reagent concentration on extraction has been studied previously (Sahuquillo et al., 1999; Maiz et al., 1997). Higher concentrations of calcium chloride were found to increase the extraction efficiency for exchangeable metals (Cd, Cu, Mn, and Ni) in contaminated soils (Maiz et al., 1997). Changing the concentration of the reducing agent (hydroxylamine hydrochloride) from 0.1 to 0.5 mol L^{-1} was reported to increase the amount of metal extracted and improve precision (Sahuquillo et al., 1999). The effect of reagent concentration for the flow system is presented in Fig. 4 and 5. No significant difference in extractability for Ca, Mn, Cu, and Zn in Step I was observed when the acetic acid concentration was increased from 0.11 to 0.22 and 0.50 mol L^{-1}, while Fe was slightly affected (Fig. 4). However, for Step II, greater amounts of Fe, Mn, and Zn were extracted when the extractant concentration was increased from 0.04 to 0.10 and 0.50 mol L^{-1} (Fig. 5). This may be attributed to a more effective attack on the more refractory crystalline forms of oxyhydroxides in the sample.

Table 3. pH of extract in a multiple-batch extraction. Sample, SRM 2710 soil; sample weight to extractant volume ratio, 0.50 g/20 mL.

	pH of extract		
pH of extractant	First batch	Second successive batch	Third successive batch
Step I, pH 3.05	3.35	3.26	3.18
Step II, pH 1.88	2.11	2.02	2.00
Step III, pH 1.86	2.07	2.03	2.01

Effect of Sample Weight to Chamber Volume Ratio

Extraction by a batch process has usually been carried out by shaking a weighed solid sample in a known volume of extractant until equilibrium is reached. The sample to extractant volume ratios (S/E) vary from 1:5 to 1:50. The BCR scheme uses a ratio of 1:40 for all steps, while the Tessier scheme uses 1:8 for the acid soluble fraction and 1:20 for the reducible fraction (Table 1). The effect of S/E ratio was studied (Maiz et al., 1997) and extractable amount of metals was found to decrease when the S/E ratio increased. This effect was more obvious for some metals than others. This is possibly caused

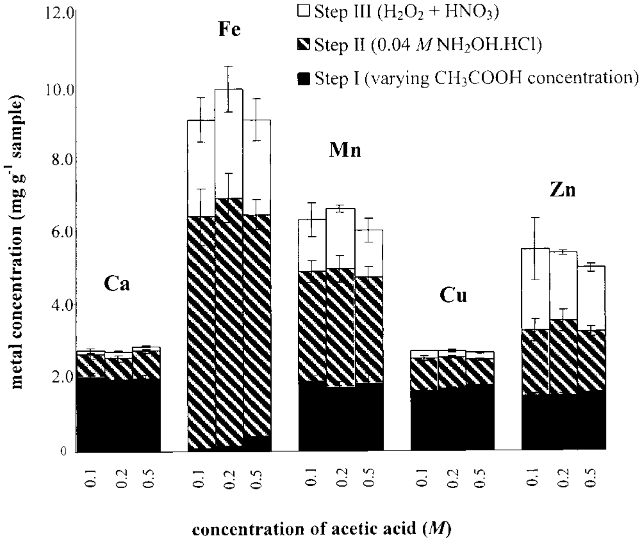

Fig. 4. Effect of varying acetic acid concentration on metal fraction distribution in soil (SRM 2710). Sample weight, 0.25 g; chamber volume, 10 mL; temperatures of Steps I, II, and III were ambient, 65°C, and 55°C, respectively.

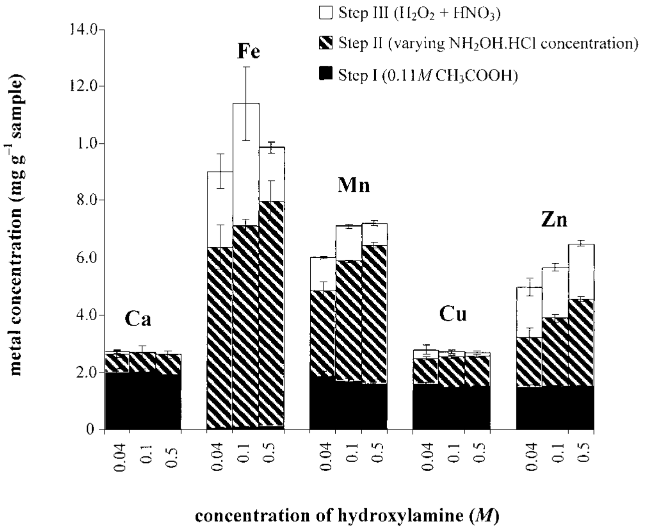

Fig. 5. Effect of varying hydroxylamine concentration on metal fraction distribution in soil (SRM 2710). Sample weight, 0.25 g; chamber volume, 10 mL; temperatures of Steps I, II, and III were ambient, 65°C, and 55°C, respectively.

by the solubility limits that different metal ions can exist with in equilibrium with other ions in the solution.

Extraction by a continuous-flow procedure is an exhaustive process and the metals in the targeted phase are totally leached, as can be observed from the extractograms. Thus, extractability can be expected to be unaffected by the S/C ratio.

Table 4 shows the effect of S/C ratio on the extractability of Ca, Fe, Mn, Cu, and Zn for the proposed continuous-flow system. The total amounts of metals extracted, which were obtained by summation of all subfractions, showed no significant difference at varying S/C ratios for all metals having high concentration levels (Table 4). Some variability was observed in the results for Ca (Step II) as attributed from relatively low concentrations and for Fe, Mn, and Zn (Step III) as a result of the variability of extraction temperature due to ineffective control. It appears that variability was higher for Steps II and III, which were performed at elevated temperatures. More precise control of temperature of extraction should lead to improved results. Extractability being unaffected by the S/C ratio is one of the advantages of the continuous-flow extraction system over the batch method. The S/C ratio, on the other hand, can affect the flow rate of extractant in the flow system. At constant power of the pump, the flow rate of extractant increased with decreasing S/C ratio (Fig. 6), especially for a chamber volume of 10 mL. This effect was due to the increase in surface area of the filter per sample weight in the bigger chamber. It can be seen that low S/C ratios can reduce extraction time significantly. However, at low S/C ratios, metal concentrations in extracts become diluted; therefore, the S/C ratio should be selected considering the concentration levels and the detection limits of the element of interest.

The detection limits for the continuous-flow approach can be somewhat poorer than for the batch method at similar S/C or S/E ratios. This occurs because larger volumes of extractant are used with the continuous flow method (160 mL compared with 10 mL in a single batch extraction for 0.25 g sample). However, since the metals are not equally distributed between the subfractions but are mostly leached in the first few subfractions, this is not as big a problem as it might initially appear. For example, in Step II (Fig. 2), metals were mostly leached in the first subfraction. The concentration of metals in the first subfraction (20 mL) would be about half of

Table 4. Extractable amounts of Ca, Fe, Mn, Cu, and Zn using continuous-flow sequential extraction at various sample weight to chamber volume ratios (S/C) for SRM 2710 soil. Detection limits for Ca, Fe, Mn, Cu, and Zn are 0.14, 0.18, 0.07, 0.03, and 0.03 mg L^{-1} extract, respectively, or 89, 115, 45, 19, and 19 μg g^{-1} soil, respectively (when metals are assumed to be equally distributed in the total extract volume of each step).

Element	Sample weight tochamber volume ratio (S/C)	Amount extracted Step I (ambient)	 Step II (65°C)	 Step III (55°C)	Sum (of three steps)
		mg g^{-1} sample ± 2 SD†			mg g^{-1}
Ca	1:12‡	1.70 ± 0.10	0.12 ± 0.05	ND¶	1.82 ± 0.15
	1:20‡	1.90 ± 0.20	0.14 ± 0.04	ND	2.04 ± 0.24
	1:20§	1.70 ± 0.10	0.11 ± 0.01	ND	1.81 ± 0.11
	1:40§	1.97 ± 0.20	0.20 ± 0.06	ND	2.17 ± 0.26
Fe	1:12‡	ND	10.0 ± 1.6	1.80 ± 0.19	11.9 ± 1.8
	1:20‡	ND	8.5 ± 0.3	2.70 ± 0.08	11.3 ± 0.4
	1:20§	ND	7.4 ± 1.4	1.20 ± 0.80	8.7 ± 2.2
	1:40§	ND	7.6 ± 0.1	1.80 ± 0.30	9.5 ± 0.4
Mn	1:12‡	19.6 ± 0.150	3.72 ± 0.09	0.45 ± 0.06	6.13 ± 0.30
	1:20‡	1.81 ± 0.37	3.83 ± 0.27	1.33 ± 0.31	6.97 ± 0.95
	1:20§	1.76 ± 0.01	3.66 ± 0.14	0.32 ± 0.04	5.74 ± 0.19
	1:40§	1.71 ± 0.01	2.93 ± 0.50	0.57 ± 0.24	5.21 ± 0.75
Cu	1:12‡	1.72 ± 0.07	0.81 ± 0.02	0.12 ± 0.01	2.65 ± 0.09
	1:20‡	1.72 ± 0.17	0.88 ± 0.07	0.13 ± 0.01	2.73 ± 0.25
	1:20§	1.57 ± 0.13	0.92 ± 0.11	0.13 ± 0.02	2.62 ± 0.26
	1:40§	1.66 ± 0.01	0.90 ± 0.07	0.14 ± 0.02	2.70 ± 0.10
Zn	1:12‡	1.67 ± 0.02	2.22 ± 0.04	1.29 ± 0.09	5.18 ± 0.11
	1:20‡	1.62 ± 0.05	2.50 ± 0.10	1.55 ± 0.07	5.67 ± 0.22
	1:20§	1.54 ± 0.09	2.06 ± 0.26	0.93 ± 0.32	4.53 ± 0.67
	1:40§	1.44 ± 0.03	1.85 ± 0.11	0.90 ± 0.20	4.19 ± 0.34

† $n = 3$.
‡ Chamber volume, 3 mL.
§ Chamber volume, 10 mL; subfraction volume, 15 mL.
¶ Not detectable.

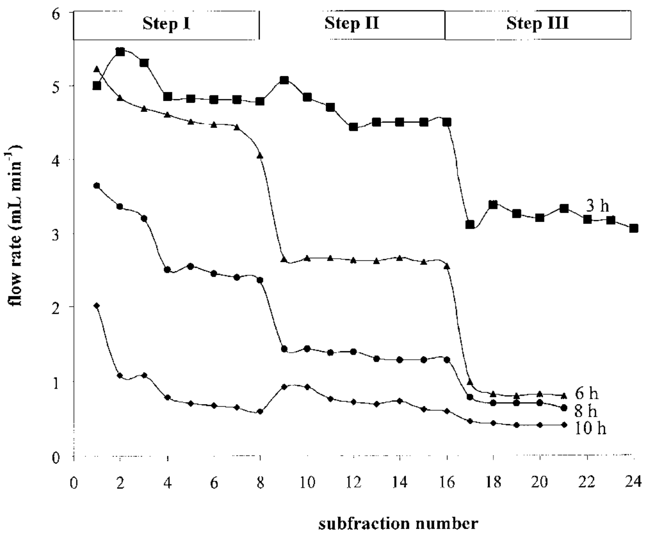

Fig. 6. Flow rate of extraction at various sample weight to chamber volume ratios. 1:12, 3-mL chamber (◆); 1:20, 3-mL chamber (●); 1:20, 10-mL chamber (▲); 1:40, 10-mL chamber (■). Sample, SRM 2710 soil; subfraction volume, 15 mL. Extraction time for three-step procedure is also indicated on each graphical plot.

that of the batch extraction using 10 mL of extractant. As a result, the detection limit of the continuous-flow extraction approach would be only twice that of the batch extraction.

As for the extraction time required, when a S/C ratio of 1:40 for the soil SRM 2710 (particle size <74 μm) was used, a three-step extraction took approximately 3 h to complete. The extraction time required for other sample types can vary due to their nature and particle sizes. For most soils and sediments, which have been analyzed in our laboratory, the time required has ranged from 2 to 6 h. Although analysis of a single sample for a continuous-flow system takes much less time than the batch system, the latter system can be more rapid when several samples (5–20 samples) are extracted in parallel. To enable simultaneous extraction of several samples in one run, a setup is currently being designed in our laboratory using a multiposition magnetic stirrer and a multichannel peristaltic pump to replace the stirrer and the pump used in this work.

The problem of flow rate changes during extraction may also be overcome by using a more powerful constant-flow pump. This will be investigated further in order to make this continuous-flow extraction approach more attractive for routine use.

The Extractogram as a Tool for the Evaluation of Chemical Associations between Elements in Solid Phases

In the proposed continuous-flow extraction system, the amount of each element in a particular phase was obtained by summation of the amounts in all subfractions of each step. Apart from the information of metal distribution in various phases, extractograms of each element as obtained by a graphical plot of extracted concentration and subfraction number can provide additional useful information. Figure 7 shows the extractograms for five metals of a reference soil sample. The appearance of the peaks of the extractograms can be used for further evaluation of the chemical association of elements in the sample. For example, double peaks were evident for Ca, Fe, Mn, and Zn (a single peak for

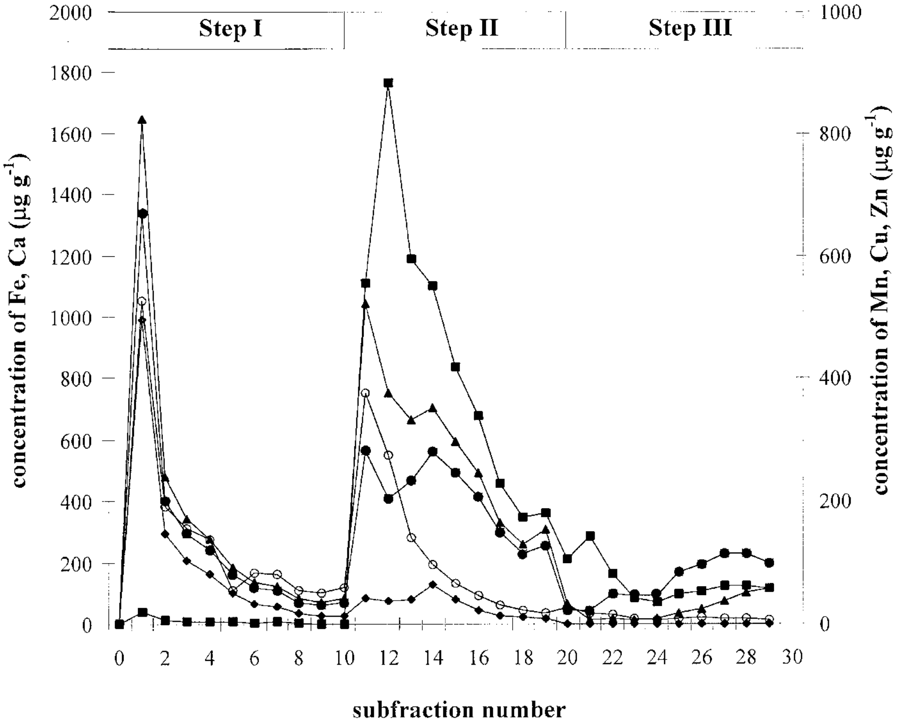

Fig. 7. Extractograms of sequential extraction of Ca (◆), Fe (■), Mn (▲), Cu (○), and Zn (●) for SRM 2710 soil. Sample, 0.25 g; chamber volume, 10 mL; subfraction volume, 15 mL. Temperature of Steps II and III were 65 and 55°C, respectively.

Table 5. Comparison of extraction results of SRM 2710 soil with those of other authors.

Elements and data from		Amount extracted					
		Step I	Step II	Step III	Residue	Total	Certified value
		mg g^{-1} sample				mg g^{-1}	
Ca	Li et al., 1995†	1.94 ± 0.15	0.22 ± 0.03	0.15 ± 0.02	10.40 ± 1.00	12.70 ± 1.00	12.50 ± 0.30
	Hall et al., 1996†	2.76 ± 0.13	0.81 ± 0.04	0.36 ± 0.02	8.40 ± 0.43	12.33 ± 0.61	
	Ho et al., 1997†	ND‡	ND	ND	ND	ND	
	This work (flow)§	2.10 ± 0.26	0.48 ± 0.26	0.20 ± 0.20	8.43 ± 1.40	11.21 ± 0.73	
	This work (batch)§	1.00 ± 0.16	0.30 ± 0.08	0.16 ± 0.04	10.50 ± 0.60	11.96 ± 0.31	
Fe	Li et al., 1995†	0.20 ± 0.09	5.15 ± 1.82	0.47 ± 0.20	28.10 ± 2.60	33.90 ± 3.20	33.80 ± 1.00
	Hall et al., 1996†	0.090 ± 0.01	9.42 ± 0.25	5.11 ± 0.04	15.94 ± 0.23	30.56 ± 0.24	
	Ho et al., 1997†	ND	ND	ND	ND	ND	
	This work (flow)§	0.13 ± 0.08	6.80 ± 1.72	1.78 ± 0.42	23.80 ± 0.48	32.51 ± 0.92	
	This work (batch)§	0.10 ± 0.04	6.30 ± 0.44	1.22 ± 0.28	27.00 ± 2.60	34.62 ± 1.33	
Mn	Li et al., 1995†	1.31 ± 0.14	4.66 ± 0.87	0.63 ± 0.04	3.83 ± 0.24	10.40 ± 0.90	10.10 ± 0.40
	Hall et al., 1996†	1.01 ± 0.01	4.52 ± 0.02	0.17 ± 0.01	3.38 ± 0.03	9.08 ± 0.04	
	Ho et al., 1997†	ND	ND	ND	ND	ND	
	This work (flow)§	1.96 ± 0.30	3.72 ± 0.18	0.45 ± 0.12	4.37 ± 1.20	10.50 ± 0.63	
	This work (batch)§	1.02 ± 0.22	4.10 ± 0.30	0.33 ± 0.04	3.29 ± 0.42	8.74 ± 0.28	
Cu	Li et al., 1995†	0.90 ± 0.06	0.70 ± 0.11	0.66 ± 0.07	0.39 ± 0.03	2.65 ± 0.14	2.95 ± 0.13
	Hall et al., 1996†	1.50 ± 0.09	0.93 ± 0.06	0.10 ± 0.01	0.44 ± 0.01	2.96 ± 0.01	
	Ho et al., 1997†	0.99 ± 0.05	0.85 ± 0.02	0.39 ± 0.02	0.43 ± 0.10	2.66 ± 0.13	
	This work (flow)§	1.58 ± 0.18	0.98 ± 0.10	0.13 ± 0.02	0.25 ± 0.08	2.94 ± 0.11	
	This work (batch)§	0.85 ± 0.16	0.80 ± 0.16	0.74 ± 0.38	0.44 ± 0.10	2.83 ± 0.23	
Zn	Li et al., 1995†	1.19 ± 0.05	2.01 ± 0.26	0.39 ± 0.03	2.86 ± 0.35	6.45 ± 0.44	6.95 ± 0.09
	Hall et al., 1996†	1.48 ± 0.01	1.74 ± 0.03	1.04 ± 0.02	2.02 ± 0.03	6.28 ± 0.02	
	Ho et al., 1997†	1.21 ± 0.06	1.12 ± 0.03	0.52 ± 0.04	3.61 ± 0.35	6.46 ± 0.09	
	This work (flow)§	1.63 ± 0.06	2.39 ± 0.26	1.38 ± 0.32	2.44 ± 0.42	7.84 ± 0.30	
	This work (batch)§	0.99 ± 0.16	2.10 ± 0.50	0.52 ± 0.08	2.98 ± 0.22	6.55 ± 0.29	

† Batch sequential extraction method.
‡ Not determined.
§ 2 ± SD, $n = 3$.

Cu) for the reducible phase (Step II). Since this step dissolves oxides and hydroxides of Fe and Mn, the double peaks may be ascribed as originating from the amorphous and crystalline phases of such materials. The existence of Zn in the reducible phase can be evaluated from the double peak of the Zn extractogram in Step II as associated with both the amorphous and crystalline phases, with a higher proportion in the crystalline phase. Copper, showing a single peak, is thought to be associated solely with the amorphous phase. The usefulness of extractogram in this aspect will be described in more details in a separate report.

Comparison of Sequential Extraction Data

The use of a certified reference material (SRM 2710) in this study enables comparison of the results of sequential extraction of the same sample reported by various authors. Although several authors have reported results of sequential extraction for SRM 2710 (Table 5), it appears that each report has used a slightly different extraction scheme (Table 6). Comparison can therefore be done on an approximate basis only.

Table 5 compares the previously reported results of batch sequential extraction of soil SRM 2710 with those obtained from this work using both continuous-flow extraction and batch extraction. The continuous-flow extraction was performed using the modified BCR scheme as described in the experimental section. The temperature of Step II was performed at 80°C because ambient temperature was found to only partially leach the reducible phases. The batch extraction was performed as described by Sahuquillo et al. (1999), with Step II performed at 80°C. Li et al. (1995) used a four-step sequential extraction, therefore their results of the exchangeable fraction and acid-soluble fraction were combined to compare with Step I of the other results. Hall et al. (1996) used a four-step extraction with two steps for the reducible fraction. The results of reducible fraction were therefore calculated from the sum of both reducible fractions. It should also be noted that Hall et

Table 6. Extraction schemes used in Table 5.

Author	Step I	Step II	Step III
Li et al., 1995	−0.5 *M* $MgCl_2$, pH 7 −1.0 *M* AcONa, pH 5 (S/E = 1:8)†	0.04 *M* $NH_2OH{\cdot}HCl$ in 25% AcOH, 96°C (S/E = 1:20)	8.8 *M* H_2O_2 in 0.02 *M* HNO_3, pH 2, 85°C (S/E = 1:16)
Hall et al., 1996	1.0 *M* AcONa, pH 5 (S/E = 1:30)	−0.25 *M* $NH_2OH{\cdot}HCl$ in 0.05 *M* HCl, 60°C −1.0 *M* $NH_2OH{\cdot}HCl$ in 25% AcOH, 90°C (S/E = 1:50)	$KClO_3$, HCl, 4 *M* HNO_3, 90°C (S/E = 1.50)
Ho et al., 1997	0.11 *M* AcOH (S/E = 1:40)	0.1 *M* $NH_2OH{\cdot}HSO_4$ in NHO_3, pH 2, ambient (S/E = 1:40)	8.8 *M* H_2O_2 in NHO_3, pH 2-3, 85°C (S/E = 1:40)
This work (flow & batch)	0.11 *M* AcOH (S/C & S/E = 1:40)‡	0.1 *M* $NH_2OH{\cdot}HCl$ in HNO_3, pH 2, 65°C (S/C & S/E = 1:40)	8.8 *M* H_2O_2 in HNO_3, pH 2, 55°C (flow), 85°C (batch) (S/C & S/E = 1:40)

† S/E, sample weight to extractant volume ratio.
‡ S/C, sample weight to chamber volume ratio.

al. (1996) extracted each step twice and combined the extracts in order to improve extractability. Ho and Evans (1997) used a three-step extraction with Step II performed at ambient temperature.

Our results for the continuous-flow method show higher values than for our batch method for almost all elements in the earlier extraction steps, indicating better extractability using the flow method. For Step I, it can be seen that the results of Hall et al. (1996) and the continuous flow method show higher values than the other reported values. This can be explained by the better extractability of the flow method and the replicate extraction used by Hall et al. (1996). The results for Step II by Ho and Evans (1997) show lower values than other results because the extraction was performed at ambient temperature. This is especially true for Zn, which was found to be associated more with the crystalline oxide phase, which was more difficult to dissolve (see previous section).

Generally, it can be said that the results from the five reports are acceptable as evaluated by comparison of the sum of all fractions with the certified values. The variability of the flow method for some elements can be rather high because the result of each fraction is obtained from summation of data of eight subfractions. Fewer subfractions are recommended for smaller variability and less FAAS measurement time if fine extractograms are not required. The variability of each fraction between different authors can be explained by the variability of the procedures used. This confirms the urgent need to establish an agreed sequential extraction procedure for better comparability.

Extraction performed in a continuous-flow manner can be more favorable than the batch counterpart for many reasons. First, extraction in a flow system can better guarantee complete leaching of the targeted phase without limitations due to the low solubility of the extractable phase in the solution medium. This is because leaching occurs continuously in a flowing stream of fresh extractant. The completeness of leaching in a batch process depends very much on the solubility of the phase of interest in the extractant under the particular operating conditions. Using the batch system, it was reported that extraction should be performed twice to improve the extractability (Hall et al., 1996). We have found better recovery using a multiple extraction compared with a single extraction at each step for most soil samples analyzed (unpublished results). This indicated that dissolution of the targeted phase for most elements was not complete in a single extraction.

Second, the pH of a batch extraction can be affected by the acid–base nature of the sample and may not be the same as the original extractant pH. Some authors (Quevauviller et al., 1993) have emphasized the need to adjust the pH of the final solution to obtain correct pH conditions for extraction. However, this is tedious and may not be strictly practiced by most users of sequential extractions. This could lead to noncomparability of results. A flow system ensures that by the end of the leaching, extraction is carried out at the nominal pH, although the pH at the beginning can also be affected by the acid–base nature of the sample.

CONCLUSIONS

Advantages of the continuous-flow extraction procedure for metal speciation were demonstrated. The procedure is simple and easy to perform because tedious procedures such as solid–liquid phase separation by centrifugation and filtration are not required. As a result, there is less risk of contamination from the experimental environment and personal procedural errors. The procedure is rapid because no equilibrium has to be established between the solid–liquid phases. A three-step extraction can be carried out within 2 to 6 h compared with 2 to 3 d for a batch method. No washing step is required in a flow extraction system because there is no cross contamination between extraction steps; if necessary in some cases, washing between steps can be simply done by flowing pure water between extraction steps.

Change in pH during extraction occurs only at the beginning of the extractant flow, therefore, extraction can be presumably considered as performed at the designated pH conditions without any need of pH adjustment during extraction. The S/C ratio does not affect the amounts of metals extracted. The S/C ratio can be adjusted considering the concentration of metals to be determined. For metals with low concentrations, a high S/C ratio can ensure that metal concentrations in extracts are at detectable levels, however with a sacrifice of a slower flow rate and extraction speed. As for the effect of extractant concentration, it was found that Step I was less vulnerable to varying concentration and the value of 0.11 to 1.0 mol L^{-1} acetic acid has little effect on extractability of metals studied. However, extraction of the reducible fraction was more sensitive to concentration change.

Extraction using a continuous-flow system still has many areas to be investigated. The problem of readsorption in this system should be much less than in a batch process because extraction times are greatly reduced, thereby allowing minimum opportunity for readsorption to occur. The study of readsorption problems is currently ongoing and will be presented in a future report. The use of extractograms to evaluate the chemical association of elements in solids is another area requiring further investigation.

ACKNOWLEDGMENTS

This work is financially supported by the Thailand Research Fund. Partial support from the Chemistry Consortium, Higher Education Development Project of the Ministry of University Affairs, and the Chulabhorn Foundation's Royal Golden Jubilee Scholarship to one of the authors (N. Tantidanai) are highly acknowledged. The authors would like to thank Prof. R.G. McLaren and Dr. H.J. Di of Lincoln University, New Zealand for their valuable comments and English corrections. Suggestions from Dirk Wallschläger and two anonymous reviewers are gratefully appreciated.

REFERENCES

Davidson, C.M., P.C.S. Ferreira, and A.M. Ure. 1999. Some sources of variability in application of the three-stage sequential extraction procedure recommended by BCR to industrially contaminated soil. Fresenius J. Anal. Chem. 363:446–451.

Hall, G.E.M., G. Gauthier, J.C. Pelchat, P. Pelchet, and J.E. Vaive. 1996. Application of a sequential extraction scheme to ten geological certified reference materials for the determination of 20 elements. J. Anal. Atom. Spectrom. 11:787–796.

Hirner, A.V. 1992. Trace element speciation in soils and sediments using sequential chemical extraction methods. Int. J. Environ. Anal. Chem. 46:77–85.

Ho, M.D., and G.J. Evans. 1997. Operational speciation of Cd, Cu, Pb and Zn in the NIST SRM 2710 and 2711 (Montana soil) by the BCR sequential extraction procedure and FAAS. Anal. Comm. 34:363–364.

Howard, J.L., and J. Shu. 1996. Sequential extraction analysis of heavy metals using a chelating agent (NTA) to counteract readsorption. Environ. Pollut. 91:89–96.

Kennedy, V.H., A.L. Sanchez, D.H. Oughton, and A.P. Rowland. 1997. Use of single and sequential chemical extractants to assess radionuclide and heavy metals availability from soils for root uptake. Analyst 122:89R–100R.

Kheboian, C., and C.F. Bauer. 1987. Accuracy of selective extraction procedures for metal speciation in model aquatic sediments. Anal. Chem. 59:1417–1423.

Leleyter, L., and J.L. Probst. 1999. A new sequential extraction procedure for the speciation of particulate trace elements in river sediments. Int. J. Environ. Anal. Chem. 73:109–128.

Li, X.D., B.J. Coles, M.H. Ramsey, and I. Thornton. 1995. Chemical partitioning of the new NIST SRMs (SRM 2709–2711) by sequential extraction using inductively coupled plasma atomic emission spectrometry. Analyst 120:1415–1419.

Lo, I.M.C., and X.Y. Yang. 1998. Removal and redistribution of metals from contaminated soils by sequential extraction method. Waste Manage. 18:1–7.

Luo, Y.M., and P. Christie. 1998. Choice of extraction technique for soil reducible trace metals determines the subsequent oxidizable metal fraction in sequential extraction schemes. Int. J. Environ. Anal. Chem. 72:59–75.

Mahan, K.I., T.A. Foderaro, T.L. Garza, R.M. Martinez, G.A. Maroney, M.R. Trivisonue, and E.M. Willging. 1987. Microwave digestion technique in the sequential extraction of calcium, iron, chromium, manganese, lead, and zinc in sediments. Anal. Chem. 59: 938–945.

Maiz, I., M.V. Esnaola, and E. Millan. 1997. Evaluation of heavy metals availability in contaminated soils by a short sequential extraction procedure. Sci. Total Environ. 206:107–115.

Nagourney, S.J., N.J. Tummillo, Jr., J. Birri, K. Peist, and J.S. Kane. 1997. Development of reference materials for the analysis of acid-extractable metals in sludge. Talanta 44:189–196.

Perez-Cid, B., I. Lavilla, and C. Bendicho. 1996. Analytical assessment of two sequential extraction schemes for metal partitioning in sewage sludge. Analyst 121:1479–1484.

Quevauviller, Ph. 1998. Operationally defined extraction procedures for soil and sediment analysis I. Standardization. Trends Anal. Chem. 17:289–298.

Quevauviller, Ph., W. Cofino, and L. Cortez. 1998. Use of reference materials in accreditation systems for environmental laboratories. Trends Anal. Chem. 17:241–248.

Quevauviller, Ph., G. Rauret, and B. Griepink. 1993. Conclusions of the workshop. Single and sequential extraction in sediments and soils. Int. J. Environ. Anal. Chem. 51:231–235.

Raksasataya, M., A.G. Langdom, and N.D. Kim. 1996. Assessment of the extent of Pb redistribution during sequential extraction by two different methods. Anal. Chim. Acta 332:1–14.

Raksasataya, M., A.G. Langdom, and N.D. Kim. 1997. Inhibition of Pb redistribution by two complexing agents (cryptand and NTA) during a sequential extraction of soil models. Anal. Chim. Acta 347:313–323.

Sahuquillo, A., J.F. Lopez-Sanchez, R. Rubio, G. Rauret, R.P. Thomas, C.M. Davidson, and A.M. Ure. 1999. Use of a certified reference material for extractable trace metals to assess sources of uncertainty in the BCR three-stage sequential extraction procedure. Anal. Chim. Acta 382:317–327.

Shan, X.Q., and B. Chen. 1993. Evaluation of sequential extraction for speciation of trace metals in model soil containing natural minerals and humic acid. Anal. Chem. 65:802–807.

Tessier, A., P.G.C. Campbell, and M. Bisson. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51:844–851.

Tu, Q., X.Q. Shan, J. Qian, and Z.M. Ni. 1994. Trace metal redistribution during extraction of model soils by acetic acid/sodium acetate. Anal. Chem. 66:3562–3598.

Ure, A.M., Ph. Quevauviller, H. Muntau, and B. Greipink. 1993. Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities. Int. J. Environ. Anal. Chem. 51: 135–151.

Usero, J., M. Gamero, J. Morillo, and I. Gracia. 1998. Comparison study of three sequential extraction procedures for metals in marine sediments. Environ. Int. 24:487–496.

Vela, L.D., R.E. Jervis, and S.S. Krishnan. 1994. Physical and chemical phenomena affecting the leachability of elements in solid wastes. J. Radioanal. Nucl. Chem. 150:45–48.